IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Michael S. Colman, et al.
Serial No.	:	09/766,161
Filed	:	January 19, 2001
For		IMPROVED RECOVERY OF LINEAR NUCLEIC ACIDS BY SALT DILUTION AND/OR REDUCED PRESSURE PRIOR TO CONTINUOUS PRESSURE DIFFERENTIAL ULTRAFILTRATION
Examiner	:	Menon, Krishnan S.
Art Unit	:	1723
Attorney Docket No.	:	MCA-538

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents P.O. Box 1450, Alexandria, VA 22313-1450 on February 13, 2004 (Date)

Kevin S. Lemack

Name of applicant, assignee, or Registered Representative

Signature

February 13, 2004

Date

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION UNDER 37 C.F.R. §1.132

I, Jack Leonard, hereby declare:

That I am a now Director of Research and Development, Life Science Research Applications, for Millipore Corporation; that from 2001-2003, I was Director of Research and Development, Genomics & Molecular Biology, for Millipore Corporation; that from 1996-2000, I was Senior Product Development Manager, Molecular Biology Group, for Millipore Corporation; that from 1995-1996, I was Manager, Laboratory Products Development and Applications, for Amicon, Inc.; and that from 1993-1995, I was a Research Biochemist, Laboratory Products Development and Applications Group, for Amicon, Inc.;

That I was a Post-doctoral Fellow of New England Biolabs, Beverly, Massachusetts;

That I am a graduate of the University of Connecticut, from which I hold a Ph.D. degree in genetics, and am a graduate of Hampshire College, Amherst, Massachusetts, from which I hold a bachelors degree in biology;

That I am a named inventor on three issued United States patents in the field of genomics and molecular biology;

That I have authored or co-authored more than ten published articles in the field of genomics and molecular biology; and

That I have reviewed the above-referenced patent application as well as the Office Action dated October 16, 2003, and I am familiar with its prosecution and the cited reference, and have made the following observations and conclusions with respect thereto.

Bussey (U.S. Patent No. 6,011,148) does not teach fractionation of different length nucleic acids or the ability to modify the selectivity of ultrafiltration for shorter species of nucleic acids by dilution alone, or the further enhancement of that selectivity with reduced vacuum pressure, as is taught by the present invention. Recovery of nucleic acids as taught by Bussey is an inherent limitation of the ultrafiltration membrane, not an effect of dilution. Bussey's stated objective is to generate pharmaceutical grade covalently closed, circular plasmid DNA suitable for gene therapy, not for improved recovery of short linear nucleic acids. Bussey is dealing with closed circular DNA (linear polymers that polymerize onto themselves) that have a different morphology than linear DNA. The present claims recite the fractionation of samples that consist essentially of linear nucleic acids, which are therefore devoid of closed circular DNA. The DNA that Bussey teaches is not linear as the Examiner indicates.

Dilution by continuous or discontinuous diafiltration as described by Bussey is for the well-known purpose of salt and contaminant removal. The diluents suggested by Bussey including water, EDTA, Tris-HCl and their mixtures are added to dilute and remove salts, not to affect the retention of nucleic acids. Furthermore, the process described by Bussey would not have accomplished the improved recovery of shorter (polymerase chain reaction) PCR products as described by the instant invention, nor would one skilled in the art be

compelled by Bussey to try to improve recovery of short PCR products using his methods, or variations thereon.

In addition, tangential flow filtration is not employed in laboratory-scale (i.e., less than one liter) ultrafiltration, and it would be highly impractical to do so. Even if one could reduce laboratory-scale tangential flow filtration to practice, it would no longer produce the effect desired in the present invention, because the salt concentration would change dramatically over the course of the filtration, and thereby undermine the basis for the enhanced separation and recovery.

Further still, the present claims recite that the samples "consist essentially of" linear nucleic acids. Accordingly, Bussey's pre-dilution step to eliminate contaminants would be redundant. The only purpose of dilution in the present invention is to change the retention properties of ultrafiltration for linear nucleic acids, not to eliminate contaminants (which are not present, or if present, are not present in an amount that would alter the characteristics of the sample). Indeed, researchers that use constant pressure differential ultrafiltration do not require dilution of linear DNA to remove contaminants from PCR samples, and there is no compelling reason for them to pre-dilute the sample as taught by Bussey.

The basis of the separation taught by Geiger ('342) is the hybridization of a short nucleic acid probe to a much longer nucleic acid target molecule. The probe molecule, if hybridized to a complementary target sequence, is effectively sequestered by the longer target molecule. This is a completely different mechanism of retention than taught by the present invention, and would behave oppositely. It is well understood by one of ordinary skill in the art that the hybridization of nucleic acid probe is favored by high salt concentration, whereas it would be unexpected that retention of small PCR products is favored by low salt concentration as taught by the present invention.

The remaining secondary references do not overcome the deficiencies of Bussey. It is noted that Simon (048) uses monovalent and bivalent cations (i.e., KCl and MgCl2) only for the purpose of sustaining the enzymatic activity of the PCR, not for the removal of contaminants by ultrafiltration, as suggested in the Office Action.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

February 12, 2004
Date

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